bio-protocol Preprint

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Droplet capture of single nuclei, cDNA sequencing and data analysis

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An abbreviated version of this protocol was published in eLIFE in Oct 2019

Stereotyped transcriptomic transformation of somatosensory neurons in response to injury

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Detailed protocol

1. Cut tissues into small pieces (1-2 mm) and incubate in RNAlater (ThermoFisher, cat# AM7021) overnight at RT
2. Remove excess RNAlater and freeze tissue pieces in a sterile microfuge tube on dry ice. Store at -80ºC until use
3. On day of 10x run: prepare homogenization buffer (250 mM sucrose, 25 mM KCl, 5 mM MgCl2, 10 mM Tris, pH 8.0, 1 µM DTT, 0.1% Triton X-100 (v/v). and cool on ice. The buffer can be prepared ahead of time and store at 4ºC for up to 6 months, except for the DTT and Triton X-100. Add those fresh on day of use.
4. Cool down all necessary equipment and solutions
5. Homogenize tissues in glass dounce homogenizer (Fisher Scientific, Cat# 357538) in 1ml of cold homogenization buffer (make fresh) 5 strokes with "loose" pestle and ~15-20 strokes with "tight" pestle … on ice
6. Filter through 40 µm cell strainer (ThermoFisher Scientific, cat# 08-771-1)
7. Transfer to microfuge tube (low bind; Sorenson BioScience, cat# 11700) and spin @ ~800g at 4o for 8 mins
8. Remove supernatant and resuspend pellet in 500 µl of PBS + 1% BSA + SUPERaseIn RNase Inhibitor (0.2 U/µ; ThermoFisher Scientific, Cat# AM2696)
9. Incubate on ice for 10-15 mins
10. Add rabbit polyclonal anti-NeuN antibody (Millipore, cat#ABN78) 1:2000 to 1:5000
11. Incubate with rotation at 4o for 30 mins
12. Spin @ ~800g at 4o for 8 mins
13. Remove supernatant and "wash" by adding 1 ml PBS + 1% BSA + SUPERaseIN RNase inhibitor
14. Spin @ ~800g at 4o for 8 mins
15. Resuspend pellet in 80 µl of PBS + 0.5% BSA + 2mM EDTA
16. Add 20 µl of anti-rabbit IgG Microbeads (Miltenyi Biotech, cat# 130-048-602)
17. Incubate at 4o 15-20 mins
18. "wash" by adding 1 ml of PBS + 0.5% BSA + 2mM EDTA
19. Spin @ ~800g at 4o for 8 mins
20. Load onto LS column (Miltenyi Biotec, cat# 130-042-401) – follow instruction from manufacturer:
    a. put column on magnetic MidiMACS separator (Miltenyi Biotec, cat#130-042-302; MACS MultiStand (cat#130-042-303), add 3 ml of buffer (PBS + 0.5% BSA + 2mM EDTA) to equilibrate
    b. resuspend nuclei pellet (from above) in 0.5-1 ml buffer
    c. Add nuclei to column
    d. Wash column with 3 ml buffer (3x)
    e. Remove column from magnet and elute in 5 ml of same buffer

21. Spin @ 500g at 4o for 10 mins
22. Remove supernatant
23. Add 1.5 ml of PBS + 1% BSA
24. Homogenize with Ultra-Turrax (Laboratory Supply Network, Inc., cat#IKA:3737001) on setting 1 for 45 sec on ice (adjust time accordingly … depending on many nuclei you have)
    a. 1st count: Stain 10 µl with trypan blue and count (using a hemocytometer) also check for clumps
25. Transfer to microfuge tube and spin @ ~800g at 4o for 8 mins
26. Remove supernatant and resuspend in desire volume with PBS + 1% BSA … volume based on the first count.
27. *Usually I try to go for 1000 nuclei/µl (if there are enough nuclei). I also assume that the first count is not that accurate (too diluted), so usually resuspend nuclei in ½ the calculated volume.*
28. Stain 10µl with trypan blue and do a 2nd count
29. Optional: do a 3rd count. This should be close to the 2nd count.

30. **Homogenization Buffer:
31. ** *Can also use EZ PREP buffer (Sigma, Cat #NUC-101) for the homogenization buffer.*

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Ryba, N. (2019). Droplet capture of single nuclei, cDNA sequencing and data analysis. Bio-protocol Preprint. bio-protocol.org/prep136.
2. Nguyen, M. Q., Le Pichon, C. E. and Ryba, N.(2019). Stereotyped transcriptomic transformation of somatosensory neurons in response to injury. eLIFE. DOI: 10.7554/eLife.49679